defects in synaptic structure and function precede, and may contribute to, the later motor neuron degeneration that is characteristic of ALS.

963-Pos Board B718
Upregulation of Glutamatergic Receptor-Channels is Associated with Cross-Modal Reflexes Encoded in Barrel Cortex and Piriform Cortex
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Associative learning is essential for cognitions. To better understand the mechanisms underlying associative learning, we examined whether the association of two signals induces a process that one signal evokes a recall of another signal, or turned around, and how glutamatergic receptor-channels are regulated to be involved in this reciprocal information retrieval (cross-modal reflex). In our mouse model, the two sensory systems, whisker-to-barrel cortex and olfaction-to-piriform cortex, were associatively activated by simultaneously stimulating whiskers and olfaction. This training procedure for 2 weeks led to odorant-induced whisker motion and whisker-induced olfaction responses. After this cross-modal reflex onset, the barrel and piriform cortices connected each other. Local filed potentials *in vivo* showed that the neurons in both barrel cortex and piriform cortex turned into processing whisker signal and odor one, respectively. The activity patterns of these cortical neurons in response to whisker signal and odorant one were distinct. These results indicate that the associative activation of barrel cortex and piriform cortex makes network neurons being able to store both whisker and odor signals as well as to recognize their differences through distinct encodings. With this reciprocal information retrieval, either of two associated signals can induce two responsive behaviors for well-organized cognitions and responses to environmental alerts. In terms of synaptic plasticity, we found that excitatory postsynaptic currents mediated by ionotropic glutamatergic receptor-channels in pyramidal neurons were upregulated in their amplitudes and frequency. By genome-wide sequencing, we observed that the upregulations of neuropilin (NMDAR accessory unit), AMPAR1 precursor, neuroligin 1~3 and tyrosine kinase in these cortical areas were associated with the cross-modal reflex and glutamate receptor-channel upregulation. [Supported by National Basic Research Program (2013CB531304 and 2011CB504405) as well as Natural Science Foundation China (30990261 and 81171033) to JHW].

964-Pos Board B719
2-Photon Imaging of Excitatory Potentials in Dendritic Spines using Voltage-Sensitive Dyes
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We developed a 2-photon imaging system that allows us to simultaneously perform glutamate uncaging and voltage sensitive dye (VSD) imaging from dendritic spines, which are the postsynaptic targets of excitatory inputs. Using this system and the intracellular fluorinated VSD di-2-AN(F)EPPTEA (Yan et al, 2012), we were able to record MNI-glutamate uncaging evoked excitatory postsynaptic potentials (EPSPs) from single spine heads that resemble mEPSPs (<1 mV at the soma) in the basal dendrites of LV pyramidal neurons. Our data suggests that in the spine heads, these EPSPs do not exceed more than 25 mV, and are attenuated by a mean factor of ~20 when they reach the soma. Interestingly, we have found no correlation between the EPSP amplitude in the spines and at the soma, which could be due to variability in the spine neck resistances. Based on this, we combined these experiments with FRAP of Alexa488, in order to estimate the spine neck resistance from the spines where we measured the EPSPs amplitudes. The time constant of equilibration of the cytosolic Alexa488, combined with the spine head volume, can be used to estimate the spine neck resistance. We used an image processing algorithm to determine the spine head volume from the 3D fluorescence distribution of Alexa488; from this, we have estimated spine neck resistances that range between 75-500 MΩ. By measuring the EPSP from a neighboring spine, we show experimentally that a spine with a neck resistance of ~75 MΩ does not fully compartmentalize the EPSP, and it can be seen by a neighboring spine ~5 um away with an amplitude > 5mV. NIH grants R01 EB001963, P41 GM103313.

965-Pos Board B720
A Multifunctional Pipette for Localized Drug Administration to Brain Slices
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We have developed a superfusion method utilizing an open-volume microfluidic device for administration of pharmacologically active substances to selected areas in brain slices with high spatio-temporal resolution. The method consists of a hydrodynamically confined flow of the active chemical compound, which locally stimulates neurons in brain slices, applied in conjunction with electrophysiological recording techniques to analyze the response. The microfluidic device, which is a novel free-standing multifunctional pipette, allows diverse superfusion experiments, such as testing the effects of different concentrations of drugs or drug candidates on neurons in different cell layers with high positional accuracy, affecting only a small number of cells. We demonstrate herein the use of the method with electrophysiological recordings of pyramidal cells in hippocampal and prefrontal cortex brain slices from rats, determine the dependence of electric responses on the distance of the superfusion device from the recording site, document a multifold gain in solution exchange time as compared to whole slice perfusion, and show that the device is able to store and deliver up to four solutions in a series. Localized solution delivery by means of open-volume microfluidic technology also reduces reagent consumption and tissue culture expenses significantly, while allowing more data to be collected from a single tissue slice, thus reducing the number of laboratory animals to be sacrificed for a study.

Magnetic Resonance Spectroscopy and Imaging

966-Pos Board B721
Optical Magnetic Imaging with Nitrogen-Vacancy Centers in Diamond
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We present recent work on developing an optical magnetic imaging system using nitrogen-vacancy (NV) color centers in diamond. The NV center is a photostable and bio-compatible sensor that can be used for both DC and AC magnetic field detection. We applied this imaging system to study DC magnetic fields produced by living cells. For AC detection, the spatial resolution and magnetic field sensitivities are further improved by use of phase encoding technique, widely used in the conventional magnetic resonance imaging.

967-Pos Board B722
Probing the Structural Topology of a Membrane Peptide in Mechcanically Aligned Lipid Bilayers using Bifunctional Spin Labeling EPR Spectroscopy
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Electron Paramagnetic Resonance (EPR) spectroscopy coupled with site-directed spin labeling (SDSL) is a powerful structural biology tool for studying the structural and dynamic properties of peptides, proteins, and nucleic acids. The most commonly used spin label for SDSL is methanthiosufonate (MTSL), however the flexibility of this spin label can introduce greater uncertainties in the EPR measurements for determining structure, side chain orientations and backbone motion of membrane protein systems. Another spin label, 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) has been found to be a useful alternative given its rigid structure, however it is very challenging to introduce this spin label into biological protein systems. The goal of this research is to develop an improved biophysical method for studying the structural and dynamic properties of membrane proteins using EPR spectroscopy that will overcome the limitations associated with MTSL and TOAC. A recently discovered bifunctional spin label (BSL) 3,4-Bis-(methanethiosulfonylmethyl)-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-1-yloxy, will be utilized. Fmoc solid phase peptide synthesis (SPPS) will be used to generate a double cysteine mutant of the 23 amino acid α-helical membrane peptide, AChR M2δ. Once labeled with BSL and incorporated into 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC) bilayers, alignment techniques utilizing EPR spectroscopy will be performed to examine the structural topology. This study will provide a structural biology tool that can be used to obtain very accurate and precise EPR measurements to answer several structural and dynamics related questions on membrane protein systems.